

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 00/01903

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/86 C12N7/01 C12N5/10 A61K39/00 A61K39/145  
A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DE 197 09 512 A (HOBOM GERD PROF DR DR) 10 September 1998 (1998-09-10)  the whole document ---	1,4,5, 12,14, 18-33
Y	WO 91 03552 A (SINAI SCHOOL MEDICINE) 21 March 1991 (1991-03-21)  figure 11; example 7 ---	1,4,5, 12,14, 18-33
Y	TAKASE H. ET AL: "Antibody responses and protection in mice immunized orally against influenza virus." VACCINE, vol. 14, no. 17/18, 1996, pages 1651-1656, XP002110225 page 1652, left-hand column, paragraph 1 --- -/-	27

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

15 June 2000

Date of mailing of the international search report

07/07/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2

NL - 2280 HV Rijswijk

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Authorized officer

Mandl, B

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/01903

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZHOU Y. ET AL.: "Membrane-anchored incorporation of a foreign protein in recombinant Influenza virions." VIROLOGY, vol. 246, 20 June 1998 (1998-06-20), pages 83-94, XP002110226 the whole document ---	5
A	ZOBEL A. ET AL.: "RNA polymerase I catalysed transcription of insert viral cDNA." NUCLEIC ACIDS RESEARCH, vol. 21, no. 16, 1993, pages 3607-3614, XP002110227 page 3607, right-hand column, paragraph 2 page 3612, right-hand column, paragraph 2 -page 3613, left-hand column, line 1 page 3614, left-hand column, paragraph 2 ---	15-17
A	WO 96 10641 A (BAYER AG ;HOBOM GERD (DE); NEUMANN GABRIELE (DE); MENKE ANNETTE (D) 11 April 1996 (1996-04-11) cited in the application the whole document ---	6-11
A	FLICK R. ET AL.: "Promoter elements in the influenza vRNA terminal structure." RNA, vol. 2, no. 10, 1996, pages 1046-1057, XP000914725 ISSN: 1355-8382 the whole document ---	6-11
A	NEUMANN G. AND HOBOM G.: "Mutational analysis of influenza virus promoter elements in vivo." JOURNAL OF GENERAL VIROLOGY 1995, vol. 76, no. 7, 1995, pages 1709-1717, XP002140118 ISSN: 0022-1317 cited in the application ---	6-11
A	PICCONE M. E. ET AL.: "MUTATIONAL ANALYSIS OF THE INFLUENZA VIRUS vRNA PROMOTER" VIRUS RESEARCH, vol. 28, no. 2, 1 January 1993 (1993-01-01), pages 99-112, XP000619019 ISSN: 0168-1702 the whole document --- -/--	6-11

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 00/01903

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PALESE P. ET AL.: "Negative-strand RNA viruses: Genetic engineering and applications." PROC. NATL. ACAD. SCI. U.S.A., vol. 93, October 1996 (1996-10), pages 11354-11358, XP000196755 page 11354, right-hand column, last paragraph -page 11356, right-hand column, paragraph F -----	5
P,X	NEUMANN G. ET AL.: "Plasmid-driven formation of influenza virus-like particles." JOURNAL OF VIROLOGY, vol. 74, no. 1, January 2000 (2000-01), pages 547-551, XP002140119 ISSN: 0022-538X the whole document -----	1,3-5, 12, 18-23, 25-31,33
P,A	FLICK R. AND HOBOM G.: "Interaction of influenza virus polymerase with viral RNA in the 'corkscrew' conformation." JOURNAL OF GENERAL VIROLOGY, vol. 80, no. 10, October 1999 (1999-10), pages 2565-2572, XP002140120 ISSN: 0022-1317 figure 1 -----	7-11

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/01903

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
DE 19709512	A	10-09-1998	NONE		
WO 9103552	A	21-03-1991	US	5166057 A	24-11-1992
			AT	126272 T	15-08-1995
			AU	636916 B	13-05-1993
			AU	6411890 A	08-04-1991
			CA	2065245 A	01-03-1991
			DE	69021575 D	14-09-1995
			DE	69021575 T	14-12-1995
			DK	490972 T	30-10-1995
			EP	0490972 A	24-06-1992
			ES	2075901 T	16-10-1995
			GR	90100639 A	30-12-1991
			JP	5500607 T	12-02-1993
			PT	95124 A	18-04-1991
			US	5252289 A	12-10-1993
			US	6001634 A	14-12-1999
			US	5578473 A	26-11-1996
			US	5854037 A	29-12-1998
			US	5840520 A	24-11-1998
			US	5786199 A	28-07-1998
			US	5820871 A	13-10-1998
			ZA	9006852 A	31-07-1991
WO 9610641	A	11-04-1996	EP	0704533 A	03-04-1996
			AU	3607695 A	26-04-1996
			EP	0783586 A	16-07-1997
			FI	971272 A	26-05-1997
			NZ	293600 A	28-01-1999

# TENT COOPERATION TREATY

## PCT

### INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>JH/m1 000520wo</b>	<b>FOR FURTHER ACTION</b>		see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. <b>PCT/EP 00/ 01903</b>	International filing date (day/month/year) <b>03/03/2000</b>	(Earliest) Priority Date (day/month/year) <b>06/03/1999</b>	
Applicant  <b>ARTEMIS PHARMACEUTICALS GMBH et al.</b>			

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

**4. With regard to the title,**



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

**5. With regard to the abstract,**



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

**6. The figure of the drawings to be published with the abstract is Figure No.**



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.



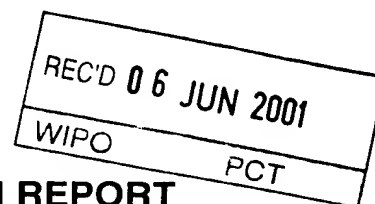
None of the figures.

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT



(PCT Article 36 and Rule 70)



9/914658

Applicant's or agent's file reference JH/ml000520wo	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP00/01903	International filing date (day/month/year) 03/03/2000	Priority date (day/month/year) 06/03/1999
International Patent Classification (IPC) or national classification and IPC C12N15/86		
Applicant ARTEMIS PHARMACEUTICALS GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.  <input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).  These annexes consist of a total of 5 sheets.
3. This report contains indications relating to the following items:  I <input checked="" type="checkbox"/> Basis of the report II <input checked="" type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application

Date of submission of the demand  02/10/2000	Date of completion of this report  01.06.2001
Name and mailing address of the international preliminary examining authority:   European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer  Marinoni, J-C  Telephone No. +49 89 2399 8563  

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/EP00/01903

**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, pages:**

1-39 as originally filed

**Claims, No.:**

1-30 as received on 22/05/2001 with letter of 21/05/2001

**Drawings, sheets:**

1/18-18/18 as originally filed

**Sequence listing part of the description, pages:**

40-54, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/EP00/01903

- ☐ the description,      pages:  
☐ the claims,      Nos.:  
☐ the drawings,      sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**II. Priority**

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:  
**see separate sheet**

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes:	Claims	1-30
	No:	Claims	none
Inventive step (IS)	Yes:	Claims	1-30
	No:	Claims	none
Industrial applicability (IA)	Yes:	Claims	1-30
	No:	Claims	none

- 2. Citations and explanations**  
**see separate sheet**



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/EP00/01903

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**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP00/01903

**Re Item II**

**Priority**

The document NEUMANN et al. 'Plasmid driven formation of influenza virus-like particles', J. VIROL., Vol. 74, No. 1, January 2000, pages 547-551 has been cited in the ISR as a P-document.

However, the claimed priority is considered to be valid. Consequently, said document is not taken into account for the establishment of the present opinion.

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. The present application relates to a genetically stable influenza virus in which one (or more) viral segments has been replaced with a ambisense RNA segment comprising the gene contained in said viral fragment and a foreign gene in opposite orientation.
2. Reference is made to the following documents:  
**D1:** DE 197 09 512 (10 September 1998)  
**D2:** WO 91/03552 (21 March 1991)
3. None of the available documents discloses such vectors:  
**D1** discloses stable influenza viruses which have all the characteristics of the viruses of the dependent claims of the present application except for the specific replacement of one or more segments by an ambisense RNAs.  
**D2** discloses unstable (see page 74, lines 6-10) influenza viruses containing one or more ambisense RNAs.  
Therefore, the subject-matter of **claims 1-30** meets the requirement of Article 33(2) PCT concerning novelty.
4. Furthermore, none of the available documents fairly suggests to replace a viral segment by a segment comprising the gene normally present in said fragment and a foreign gene specifically in ambisense orientation.  
Therefore, the subject-matter of claims 1-30 meets the requirements of Article

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/EP00/01903

33(3) PCT concerning inventive step.

**Re Item VIII**

**Certain observations on the international application**

**Claims 29, 30, 32 and 33** are partially or completely directed to methods of treatment of the human/animal body. No unified criteria exist in the PCT concerning this type of claims. It is noted that the EPO, for example, does not allow such claims.

PCT/EP00/01903

JH/ml

Artemis Pharmaceuticals GmbH

21 May 2001

**Claims**

1. A recombinant influenza virus for high-yield expression of incorporated foreign gene(s), which is genetically stable in the absence of any helper virus and which has eight viral RNA segments, wherein at least one of the regular viral RNA segments is replaced by an RNA molecule (ambisense RNA segment), said ambisense RNA segment containing one of the standard viral genes in sense orientation and a foreign, recombinant gene in anti-sense orientation, or *vice versa*, covalently linked to each other and in overall convergent arrangement.
2. The recombinant virus according to claim 1, wherein in the ambisense RNA molecule said foreign recombinant gene is covalently bound to one of the viral genes, while the original vRNA segment coding for the same gene is deleted from the recombinant virus by way of specific ribozyme cleavage.
3. The recombinant influenza virus according to claims 1 and 2, wherein one or more of the regular viral RNA segments, differing from said at least one ambisense RNA segment, comprises a vRNA encoding a foreign gene, preferably one or more of the regular viral RNA segments has (have) been exchanged for a vRNA encoding a foreign gene.
4. The recombinant influenza virus according to claim 3 in which one or both of the standard glycoproteins hemagglutinin and neuraminidase have been exchanged into foreign glycoprotein(s) or into fusion glycoproteins consisting of an anchor segment derived from hemagglutinin and an ectodomain obtained from the foreign source, viral or cellular, or in which such recombinant glycoprotein has been inserted as a third molecular species in addition to the remaining standard components.

5. The recombinant influenza virus according to claims 1 to 4, in which the terminal viral RNA sequences of one or more of the regular segments and/or of the at least one ambisense RNA segment, which are active as the promoter signal, have been modified by nucleotide substitutions in up to five positions, resulting in improved transcription rates of both the vRNA promoter as well as the cRNA promoter as present in the complementary sequence.
6. The recombinant influenza virus of claim 5, wherein the 12 nucleotide conserved influenza 3' terminal sequence has been modified by replacement of one to three nucleotides occurring in said sequence at positions 3, 5 and 8 relative to the 3' end by other nucleotides, and/or wherein the 13 nucleotide conserved influenza 5' terminal sequence has been modified by replacement of one or two nucleotides occurring in said sequence at positions 3 and 8 by other nucleotides.
7. The recombinant influenza virus of claim 6, wherein the replacements in the 3' terminal nucleotide sequence comprises the modifications G3A and C8U.
8. The recombinant influenza virus of claim 7, wherein the replacements in the 3' terminal nucleotide sequence comprises the modifications G3A, U5C and C8U, or G3C, U5C and C8G.
9. The recombinant influenza virus of claim 8, which comprises a 3' terminal nucleotide sequence of 5'-CCUGUUUCUACU-3'.
10. The recombinant influenza virus of claims 6 to 9, wherein the 5' terminal nucleotide sequence comprises the modifications U3A and A8U resulting in a 5'-terminal sequence of 5'-AGAAGAAUCAAGG.

11. The recombinant influenza virus according to claims 1 to 10, which is a recombinant influenza A virus.
12. The recombinant influenza virus according to claims 1 to 11, in which the foreign gene(s) in ambisense covalent junction with viral gene(s) code for proteins and/or glycoproteins which are secreted from cells infected with the recombinant virus.
13. The recombinant virus according to claims 1 to 11, in which the foreign gene(s) in ambisense covalent junction with viral gene(s) code for proteins or artificial polypeptides designed to support an efficient presentation of inherent epitopes at the surface of infected cells, for stimulation of a B cell and/or T cell response.
14. A method for the production of recombinant influenza viruses as defined in claims 1 to 13 comprising
- (a) RNA polymerase I synthesis of recombinant vRNAs *in vivo*, in ambisense design,
  - (b) followed by infection with an influenza carrier strain constructed to include flanking ribozyme target sequences in at least one of its viral RNA segments which is (are) to be replaced by the ambisense segments of step (a), and
  - (c) thereafter selective vRNA inactivation through ribozyme cleavage.
15. A pharmaceutical composition comprising a recombinant influenza virus according to claims 1 to 13.
16. Use of a recombinant influenza virus according to claims 1 to 13 for preparing a medicament for vaccination purposes.
17. The use according to claim 16, wherein the medicament
- (a) is suitable against influenza and/or against other infections;

- (b) is present in form of inactivated preparations; and/or
- (c) is present in form of live recombinant viruses.

18. Use of a recombinant influenza virus according to claims 1 to 13 for preparing agents for somatic gene therapy.
19. Use of a recombinant influenza virus according to claims 1 to 13 for preparing agents, for transfer and expression of foreign genes into cells infected by such viruses.
20. Use of a recombinant influenza virus according to claims 1 to 13 for preparing agents for transfer and expression of RNA molecules into cells infected by such viruses.
21. The use of claim 20, wherein the RNA molecules to be expressed are antisense sequences or double-strand sequences relative to the target cell cellular mRNA molecules, and/or the agent is suitable for sequence-specific gene silencing, preferably by antisense RNA or RNA interference mechanisms.
22. The use according to claims 18 to 21, wherein the agents are applicable in *ex vivo* and *in vivo* application schemes.
23. A method for the production of proteins or glycoproteins which comprises utilizing a recombinant influenza virus according to claims 1 to 13 as expression vector.
24. The method of claim 23, wherein the production is performed in cell culture cells or in fertilized chicken eggs.
25. A method for preventing and/or treating influenza which comprises administering an effective amount of a recombinant influenza virus according to claims 1 to 13 to the mammal to be treated.

26. A method for somatic gene therapy, which method comprises subjecting the organism to be treated with a recombinant influenza virus according to claims 1 to 13.
27. A method for transfer and expression of foreign genes into cells, and for transfer and expression of RNA molecules into cells, which method comprises infecting the cells with a recombinant influenza virus according to claims 1 to 13.
28. Use of a recombinant influenza virus according to claims 1 to 13 for preparing agents for autologous immunotherapy.
29. A method for an immunotherapy which comprises *ex vivo* infection of immune cells with a recombinant influenza virus according to claims 1 to 13, and introduction of the transduced cells into the patient.
30. A method for the induction of antibodies which comprises utilizing a recombinant influenza virus according to claims 1 to 13 as an immunogen.